

Scientific Abstract

Ischemic heart disease (IHD) due to atherosclerosis is the most common underlying cause of cardiovascular disability and death in the US and Western World. The American Heart Association (AHA) estimates that 12.6 million Americans have IHD, and over 500,000 die from the disease every year. The lifetime risk of developing IHD after age 40 is 49 percent for men and 32 percent for women. The primary symptom of IHD is angina pectoris. A number of approved medications including nitroglycerin, beta-adrenergic blocking agents, and calcium-channel blocking agents can improve symptoms by reducing myocardial oxygen demand, but no currently available medication can restore the compromised arterial blood supply. Invasive therapies such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) are reserved for high-risk patients and patients unresponsive to standard medical therapies.

Medical therapies for IHD are generally successful in controlling the symptoms of disease, but even optimal therapeutic regimens do not prevent the eventual worsening of disease. One new approach to treatment of IHD is therapeutic angiogenesis which comprises therapies capable of induction of growth of new blood vessels in the coronary vasculature. Such an approach is the basis for this clinical trial; Ischemic heart disease not amenable to coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI).

The body produces at least two forms of mitogen for hepatocytes, namely Hepatocyte growth factor (HGF) and Hepapointin (HPO), a truncated form of HGF (five fewer amino acids than HGF). A wide variety of animal models of acute and chronic liver disease have demonstrated that HPO prevents or reverses liver injury and have a potential role in patients with liver disease. Human studies have been conducted with HPO in the US, in which the safety of HPO was demonstrated with IV doses of up to 100 mg.

HGF is a 728 amino acid protein that is well characterized and is comprised of a heterodimer including a heavy chain and light chain component. The protein is excreted into extra cellular fluid after removal of the signal peptide comprising 31 amino acids. It has been shown that secreted or non-secreted HGF is expressed in almost all the normal tissues, with serum HGF concentration in the range of 0.3 ± 0.1 ng/mL in healthy volunteers and non-secreted HGF concentration is 30~40 ng/mg protein in human blood vessels. Consistent with in vitro findings that hypoxia down regulates vascular HGF production, vascular HGF concentration in the diseased segments of vessels from patients with arteriosclerosis obliterans has been reported to be significantly decreased and accompanied by a marked reduction in HGF mRNA. Additionally it has been shown that the c-met receptor (HGF specific receptor) distribution and expression level is increased in the region surrounding infarcted myocardium.

Although HGF was originally identified as a potent mitogen for hepatocytes, HGF is known to be involved in proliferation, mobility and morphogenesis of various cells, and also considered to be a humoral mediator of epithelial-mesenchymal interactions during embryonic development and organogenesis. Hepatocyte growth factor's multi-functional activity has great potential as a therapeutic agent to promote or accelerate tissue repair and organ regeneration after injury and dysfunctions in chronic disease conditions.

Additional studies have demonstrated the potential to induce myocardial angiogenesis in animal models of cardiomyopathy, infarcted myocardia and non-infarcted myocardia.

The nature of HGF is similar to that of VEGF in that it stimulates the growth of endothelial cells, but is different from acidic FGF and basic FGF that show a growth stimulation effect also on smooth muscle cells and fibroblast cells. It is hypothesized that the localized expression of HGF produced from the AMG0001 could contribute directly to the proliferation of endothelial cells and the migration of vascular smooth muscle cells. It is important to note that HGF induces migration, not proliferation, in smooth muscle cells. The proliferation of smooth muscle cells in angiogenesis is known to cause non-healthy vessels due to inappropriate vasoconstriction. Hence, HGF appears to be a more promising choice than VEGF as an agent for therapeutic angiogenesis.

Therapeutic angiogenesis is considered a direct way to treat IHD by improving blood perfusion to the ischemic areas in the heart. HGF's many biochemical and physiological activities are believed to influence angiogenesis:

- The specific receptor for HGF, c-met, has been reported to be up-regulated in response to hypoxia in a myocardial ischemia model, which may enhance the angiogenic activity of HGF.
- HGF has been postulated to promote angiogenesis as a result of a combination of direct effects on endothelial cells and indirect effects, including paracrine up-regulation of VEGF, on vascular smooth muscle cells.
- Since decrease in endogenous vascular HGF is noted in ischemic tissue, and exogenous HGF is known to enhance expression of HGF itself as well as expression of its receptor, c-met, HGF gene transfer may enhance endogenous expression of HGF and c-met.

AnGes-MG, Inc. is currently evaluating HGF in patients with severe peripheral arterial disease (PAD) enrolled into the phase I/II study (AMG001-JN-100), *Gene Therapy for Therapeutic Angiogenesis in Patients with Peripheral Arterial Diseases (Arteriosclerosis obliterans and Buerger's Disease) using Plasmid Encoding Hepatocyte Growth Factor*. This study is being conducted at a single center (Osaka University) in Japan under ICH GCP compliance. The study was designed to include 2 stages. Stage I evaluated plasmid HGF in 6 patients and is completed. Stage II is evaluating 2 doses of plasmid HGF and enrollment of the total 16 patients planned is completed.

The initial phase I/II study for IHD will have 2 stages. The first stage of this trial will be a dose-escalating safety study evaluating sequentially 4 doses of HGF DNA Plasmid (0.4 mg, 0.8 mg, 1.5 mg, and 4.0 mg) administered by intra-myocardial injections. In stage I of this trial, 8 patients will be evaluated at each dose level; 6 patients will receive plasmid HGF and 2 patients will receive placebo. Escalation of dose will depend upon the occurrence of defined dose limiting toxicities such that if greater than 2 dose limiting toxicity occurs, no higher doses of plasmid HGF will be evaluated. Three doses with acceptable safety will be compared to placebo in stage II section of the clinical study. In

stage II of the clinical study up to 60 patients will be randomized to either placebo or one of 3 doses of plasmid HGF. It is anticipated that the stage II design of this study will assess the safety of the three doses and provide some initial data regarding the effect of plasmid HGF on improved perfusion in the ischemic area of the heart. Results from this phase I/II trial will help determine the design of additional trials to further evaluate the safety and clinical effect of this gene transfer agent in IHD patients.

The results of this trial will be to establish a base of clinical information that will allow AnGes to properly design the next phase of this clinical development program.